

K/1 : NERAAL R. ET COL. : ENZYMATIC BREAKDOWN OF ADDED TRIPOLYPHOSPHATE AND DISPHOSPHATE IN MEAT.

QUESTION DE M. NILSSON :

What do you think is the contribution of myokinase, which transform two ADP to one ATP and one AMP.

As this enzyme is very heat stable and inhibited by chloride it might be of importance. Perhaps myokinase and your ADase the same enzyme.

REPOSE :

DPase is also called inorganic pyrophosphatase. There are several DPases of which some are soluble and some are not. These enzymes have been studied in isolated form. From their pH-optimums we found that the soluble DPases have the largest post mortem activity. If these enzymes also have myokinase activity is unknown to us.

QUESTION DE M. FROUIN :

Avez-vous réussi à obtenir une contraction avec l'Actomyosine ou la Myosine des viandes post rigor mortis par addition d'ATP ?

REPOSE :

By the addition of ATP in absence of NaCl, we have observed a contraction of the meat, and we suppose this is due to the contraction of actomyosin. In the presence of NaCl, however, the coupling of the actin to myosin during the contraction cycle is probably inhibited, which causes a repulsion instead of a contraction of the fibers.

QUESTION DE M. RANKEN :

The pH of a 1 % solution of sodium diphosphate is about 9-10 but 0.3% of sodium diphosphate in meat only changes the pH by about 0.2 units Do you think that this very small change in pH in the meat can explain the large effect of the added phosphate ?

REPOSE :

We think that the pH change only plays a minor role for the effect of DP on the WHC of meat. We think that the dissociation of actomyosin,

which is also achieved by the addition of NaCl to slaughterwarm meat (through the presence of ATP) is most important.

ATP, however, probably acts by a different mechanism than DP does, namely, merely by binding to the hydrolysis site of myosin. This causes an immediate coupling of actin from the actin binding site.

QUESTION DE M. FROUIN :

Je suis tout à fait d'accord avec vos résultats, et j'ai aussi observé que les polyphosphates apportent un pouvoir de rétention d'eau qui subsiste après leur disparition.

J'ai rencontré le même phénomène dans mes études sur le calcium ionisé. On constate aussi que les protéines musculaires peuvent fixer beaucoup plus d'eau qu'elles n'en fixent, et que plus l'on met d'eau avec les protéines de viande, plus elles en fixent et plus il en reste après les traitements que l'on fait suivre après.

D'autres exemples nombreux pourraient être cités.

Tout se passe comme si ces protéines avaient une sorte de mémoire des conditions passées lorsqu'elles fixent l'eau.

Ceci m'amène à formuler l'hypothèse suivante :

Dans les viandes mûries, l'eau et les protéines forment un système semi cristallin comparable aux cristaux liquides, et que ce système est stable, plus exactement assez stable.

Les polyphosphates, les autres additifs et la technologie etc... forment à un instant donné ce système cristallin, une fois ce système formé comme il est stable, la décomposition des additifs est sans grande importance sur le pouvoir de rétention d'eau.

Ceci, bien sûr, tant que l'on ne se place pas dans des conditions sévères détruisant ce système cristallin eau-protéines.

Que pensez-vous de cette hypothèse ?

REPONSE :

As already said, we think that the lack of coupling of dissociated actin and myosin caused by the repulsive forces of NaCl will conserve the water holding properties of the meat treated with inorganic polyphosphates or ATP. The dissociated actomyosin system would only be able to contract in the presence of ATP without the addition of NaCl.

QUESTION DE M. VENDEUVRE :

Man weiss aus der Praxis,

- 1) dass man bei der Kochschinkenherstellung mit TP eine höhere Ausbeute als mit DP erzielen kann,
- 2) dass bei Brühwurst umgekehrt, mit DP bessere Ergebnisse im Hinblick Ausbeute erzielt werden können. Wie können Sie das anhand Ihrer neuen Ergebnisse erklären ?

Heisst es, dass DP eine grössere Rolle für das Inlösungsgehen der Muskeleiweisse als für die Quellung und die Wasserbindung spielt ?

REPOSE :

As previously said, we think that TP dissociates actomyosin first after having been hydrolyzed to DP and monophosphate. This we think is due to the fact that TP will probably not bind to the actin binding site of myosin and thereby replace actin. This is, on the other hand, achieved by DP. The molar amount of DP is larger in a certain percent of added DP than in the same amount of added TP, and will therefore give a better effect.

In ham, we think that other factors play a more heavy role. Here, the sequestration of divalent cations may be of primer importance. This is achieved better with TP than with DP. Anyway, in this case, many questions still remain unanswered.

We think that the larger swelling of the protein structure following the dissociation of actomyosin is one of the main reasons for the increase in WNC of the meat. By this process, free myosin and actin are being produced. These proteins are known to be much more soluble than the undissociated actomyosin. Subsequently, also an increase in protein solubilization will follow.

K/2 : ANDO N. ET COL.: SOME OBSERVATIONS ON THE WATER-HOLDING CAPACITY OF MEAT.

QUESTION DE M. MOTHERHILL

Why did you study pure myosin instead of actomyosin which is what occurs in muscle, post rigor ?

REPONSE :

This is a basic investigation, and at the present stage myosin can be obtained in most pure state ; but there is much more difficulties in preparing actomyosin in quite pure state, and so to make accurate quantitative observations is much harder in actomyosin.

This is the reason.

QUESTION DE M. HOFMANN :

Welche Methode der Bestimmung des Wasserbindungsvermögens haben Sie verwendet ? Sie haben diesbezüglich nur auf frühere Literatur verwiesen, so dass die angewendete Methode aus der Arbeit nicht hervorgeht.

REPONSE :

It takes much time to tell the method of determining the water-holding capacity of meat used in our experiments, so please read it in the reference given in the text. If you want to get the preprint please contact me after this session privately.

K/5 : FROUIN A. ET COL. : COMPOSITION DU PIGMENT DES VIANDES SALEES.

QUESTION DE M. LEDWARD :

Have the authors any information on the electron spin resonance (ESR) spectrum of the ham pigment they describe in their paper ?

REPONSE :

Je n'ai pas fait de mesure, n'ayant pas d'appareil pour cette mesure mais, TORLAGIS et d'autres auteurs ont fait cette mesure et trouvé l'absence d'électron non apparié. Le schéma proposé correspond aux solubilités apparemment électroniques.

Cadre très bien avec les études de la dynamique de la coloration effectuées par JAY-FOX-AKERMANN et tout ce que j'ai trouvé dans la bibliographie citée

QUESTION DE M. VENDEUVRE :

Vous avez montré que les substances réductrices pouvaient avoir un comportement très différent pour la formation du nitrosohème. Cela veut-il dire que l'on peut envisager pour le pigment de la viande fraîche :

- 1) une corrélation élevée entre la vitesse de méthémoglobinisation et la MNA (apportée exclusivement par l'ascorbate) ?
- 2) une absence de corrélation entre la vitesse de méthémoglobinisation et la MNA (apportée exclusivement par la cystéine) ?

REPONSE :

Je pense comme FOX-AKERMANN 1er stade : Meth No à partir No les réducteurs ne jouent pas tellement.

Relation avec pouvoir réducteur complexe : stade Met intermédiaire.

QUESTION DE M. RATCLIFF :

How does your proposed scheme for the reactivities of NO and the prosthetic groups of myoglobin, account for the observation that in Wiltshire Bacon which is processed at low temperature (< 40°C), only 50 % of the total haem pigment is extractable in acetone (by Hornsey's method).

REPONSE :

1. Electrophorèse avec extraits acétoniques montrent absence de protéines si réaction avec ascorbate - pas de même avec cystéine
2. A basse température probablement peu de réducteurs libérés, autres procédés d'extraction.

QUESTION DE M. ROSKIS :

Selon les auteurs américains, le pigment de la salaison des viandes ne serait pas un véritable composé nitrosé, mais tout simplement un composé d'addition (par fixation) du NO. Ils le désignent par ailleurs traditionnellement par : " Nitric oxide myoglobine" (NO-Mb), contrairement à la nomenclature française qui parle d'un "nitroso Mb".

Comment concilier ce point de vue avec les considérations que vous venez d'esquisser ?

Quant aux risques de formation de nitrosamines, il semble que les derniers travaux en la matière retiennent plutôt la possibilité de cette formation par réaction du nitrite avec des substances à groupements aminés des tissus musculaires de la viande et non pas avec celles des pigments myoglobiniques. Qu'en pensez-vous ?

REPOSE :

Second point tout à fait d'accord.

Stabilité NO peu propice à simple addition - Composé + Fe<sup>++</sup> + NO stable à peu près en présence d'air ! à rapprocher du stade NO Meth réversible.

NO Fe<sup>++</sup> non réversible d'après majorité auteurs et pratique.

K/6 : OCKERMAN H.W. ET AL. : THE INFLUENCE OF NITRITE ON CURED PORK FLAVOUR.

QUESTION DE M. RUSSWURM :

1. Was there any trend in the development of the amount of area under the four peaks in the head space chromatograms during storage ?
2. Have you tried to concentrate the head space gas before chromatography to get some of the many other components in the head space gas ?

REPOSE :

1. The head space analysis in the experiment was only made after processing and therefore we have little information currently on its alteration with time. The few samples we did analyse with time indicated that the major alteration was instability
2. We have done some work on head space concentration and of course if this is combined with more sensitive procedures many more than four peaks can be obtained .

QUESTION DE M. HOFMANN :

Da in Ihrer Arbeit der "Aroma Panel score" eine bedeutende Rolle spielt, möchte ich Sie bitten, diesen Begriff etwas näher zu erläutern. Ist es richtig, dass dieser Begriff im umgekehrten Sinne zu verstehen ist wie etwa die "Aroma-Intensität" ? Denn Sie haben festgestellt, dass die Proben, deren Kurven am niedrigsten lagen, am besten beurteilt wurden.

REPOSE :

The panel was asked to evaluate (fig. 2 and 4) rancid odour and score it as follows,

1. No rancid odour,
2. Pronounced rancid odour.

K/7 : SKJELKVAALE R. ET AL. : THE EFFECT OF SODIUM NITRITE ON THE ORGANOLEPTIC PROPERTIES OF PROCESSED MEAT PRODUCTS.

QUESTION DE M. MOERMAN :

Is it correct to draw such a general conclusion, concerning the preference of the test of meat products with and without nitrite based on a small panel.

it is known that specialized panels often have an other preference than the consumers.

REPOSE :

What we said about the preference is only an indication that the products without nitrite added were of good quality. Our main conclusions are that a trained panel find differences in the products investigated and that the differences are found to be small. I think that is almost in agreement with the experience gained in Norway during 1973 in products where nitrite is forbidden. I agree with you that a preference test only can be correctly performed by a large number of tasters of more than 100-200, and outside the laboratories.

QUESTION DE M. GREENBERG :

Have you done any analyses for nitrogen oxide levels in finished smoked meats prepared without nitrite and/or nitrate ?

REPOSE :

My answer is no, we have not done analysis of nitrogen oxide. We analysed the residual level of nitrite, which data are found in our paper, page 1508, table 1. The data for nitrite levels in products not added with nitrite are not included in the table, but the amount of nitrite found was zero.

QUESTION DE M. NILSSON :

1. What indications do you have that the results shown in Fi. 1 are of importance for the aroma of bacon.
2. Which type of substances are found in the peaks carbonylics fatty acids or other types ?
3. Do you not think that the head space of bacon is much more complicated than the chromatograms show ?
4. Do you think that  $\text{NO}_2$  form substances necessary for the cured flavour or is the effect just to inhibit rancidity ?

REPOSE :

1. None. Our purpose here was to try to detect any major volatile chemical alteration. We did use the nose as a post detector and some panel members thought they could detect part of specific odour but this evaluation was very crude and consequently.
2. We did not report on it most of them are short chain aldehydes.
3. No doubt about it there are many more than these four compounds which are in smaller concentrations.
4. I think both effects are present. Initially there is an alteration in flavour but rancidity become more important as storage time increases.

COMMENTAIRES DU Docteur RHODES S'APPLIQUANT A K6 et K7 :

1. Involvement of mitochondrial enzyme activity in the curing reaction.

In earlier papers presented to the European Meetings, mitochondrial enzymes have been implicated in the curing reaction. Recent evidence from the Meat Research Institute has shown this to be incorrect. The mitochondria of fresh pork are rapidly destroyed by the action of salt and nitrite and the curing reaction is unaffected in pork after storage which destroys the mitochondrial structure.

2. The production of cured flavour.

The level of nitrite in Whiltshire type brines has been related to the development of cured flavour and the disappearance of salt pork flavour. The changes in the two flavours are inversely related, and cured flavour increases in intensity as the nitrite level in the brine is increased up to 1000 ppm. above this level little further increase occurs. Cured pork flavour is delicate and is recognised with difficulty even by trained taste panels. Summarising published information reveals that about one third of judgements comparing the flavour of salted pork and cured pork are in error ; in our experience this is not due to individual sensitivity.



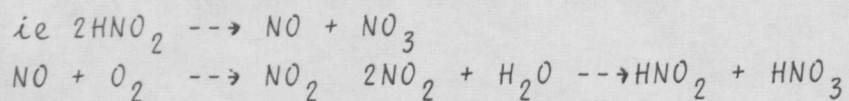
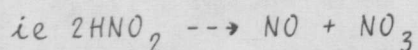
K/8 : HERRING H.K. : RECENT STUDIES ON THE ROLE OF SODIUM NITRATE  
IN CURED MEAT PRODUCTS.

QUESTION DE M. GARDNER :

In the paper, you have observed the formation of nitrate from nitrite in bacon. Would you like to comment on how this occurs ?

REPONSE :

1. Disproportion of nitrous acid.
2. Cyclic oxidation-reduction process



3. Also, Mr. Mohler has observed that there is conversion of nitrite to nitrate which occurs with autocatalytic oxidation of the iron in myoglobin.

QUESTION DE M. RANKEN :

You state that ascorbate reduced or eliminated NP formation. Is this due to reduction in the residual nitrite level, or is there a direct inhibition of nitrosation ?

REPONSE :

The mechanism is unclear, but I'm sure is being vigorously pursued in many laboratories.

QUESTION DE M. PIVNICK :

In non-sterile products such as wieners and bacon that may be contaminated during slicing, packaging, etc, can one attribute inhibition of *C. botulinum* to Perigo effect without considering the possible antagonistic effect of the non-botulinal bacteria towards *C. botulinum* ?

REPONSE :

As you pointed out in your paper, there are great many unknown factors which contribute to botulinal safety of non-sterile cured meats.

QUESTION DE M. HOFMANN :

Ich vermute, Sie haben für die Nitritbestimmung das Griess'sche Reagens verwendet, wie dies allgemein der Fall ist. Dabei stellt man eine Abnahme des Nitritgehaltes während der Lagerung und Erhitzung fest. Das ist allgemeiner bekannt. Haben Sie einmal ein anderes Nitritreagens angewendet und sind Sie dabei zu den gleichen Ergebnissen gekommen ?

Wir haben vor kurzem ein neues Nitritreagens angewendet, wobei sich sehr überraschend zeigte, dass der Nitritgehalt entgegen den bisherigen Erfahrungen weder bei der Lagerung noch bei der Erhitzung in Brühwürsten und Schinken abnimmt. Diesen Befund müsste unbedingt weiter nachgegangen werden !

QUESTION (in english)

I believe you also used for  $\text{NO}_2$  determination the GRIESS reagent. We all know that a decrease of  $\text{NO}_2$  occurs during storage and heating. Have you ever tried an other reagent and if yes, which were the results ?

Not long ago, we tried a new reagent with the surprising result that the nitrite content did not decrease either during storage or during heating in ham or cooked sausage. Should be continued these experiments.

No, the determinations were made only with GRIESS reagent. Therefore, I don't know if other reagents would give different analytical results.

Some research laboratories in the U.S. are now studying different analytical methods for nitrite and find different reagents give different results for nitrite concentrations.

QUESTION DE M. NILSSON :

Could you give us examples of lowest  $\text{NO}_2$  levels for having safe products and the statistical evidences for choosing these levels ?

REPONSE :

In Wieners, 100 ppm added nitrite was shown to have an inhibiting effect during 56 days incubation at  $27^\circ\text{C}$  and after inoculation with 620 spores per gram.

In ham (canned comminuted perishable product) inoculated with  $90^\circ\text{C}$ . botulinum spores per gram, no toxin had developed after storage for 168 days incubation when 200 ppm sodium was added.

In bacon, no C. botulinum toxin was detected when formulation was with 170 or 340 ppm after storage at  $21^\circ\text{C}$  for 3 months.

Storage at  $7^\circ\text{C}$  also protects the product, even with high inoculum levels, but we feel this cannot always be relied upon in the real world.